

Detection of *icaAD* Gene and Biofilm Formation in *Staphylococcus aureus* Isolates from Wound Infections

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Abstract

Wound infections are a common cause of staphylococcal infections. An ability of *S.aureus* is to adhere and form biofilm on host surfaces. Biofilm is an exopolysaccharide, a slime matrix around multiple layers of cells and is mediated by expression of the *icaADBC* operon. The present study evaluated the biofilm forming capacity and the presence of *icaAD* gene among *S.aureus* isolated from wound infections. Slime production assay was performed by cultivation on Congo Red Agar plate. In addition, Quantitative biofilm formation determined by microtiter plate assay PCR method used for detection of *icaAD* gene. Fifty strains were identified, 54% of the isolates produced black colonies on CRA plate, 52% were positive biofilm forming, and all strains carried the *icaAD* gene. Regarding the ability of *S.aureus* to form biofilms helps the bacterium to survive hostile environments within the host, suggests that biofilm production is a risk factor for infection. It is important in rapid diagnosis and treatment biofilm forming strains, because biofilm formation may lead to increased antimicrobial resistance and create a significant impediment to wound healing.

Keywords: *Staphylococcus aureus*, *Wound infection*, *Biofilm*

Introduction

Staphylococcal infections are a major source of patient morbidity and implant failure. *S.aureus* is the most important pathogen in the genus and is also the most important nosocomial pathogen of surgical wounds (1). Staphylococcal wound infections can occur in the patients after a surgical procedure or after trauma, with organisms colonizing the skin introduced into the wound. The virulence of *S.aureus* is associated with its ability to produce toxins and other extracellular factors, ability to adhere and form biofilm on host surfaces, finally resistance to phagocytosis (2, 3).

Biofilm is an exopolysaccharide, a slime matrix around multiple layers of cells (4, 5). Biofilm

formations is considered to be a two step process in which the bacteria first adhere to a surface mediated by a capsular antigen, namely capsular polysaccharide/adhesin (PS/A), followed by multiplication to form a multilayered biofilm, which is associated with production of polysaccharide intercellular adhesin (PIA).

The intercellular adhesion (*ica*) locus consisting of the genes *icaADBC* encodes the proteins mediating the synthesis of PIA and PS/A in staphylococcal species (6-8). Among *ica* genes, the *icaA* and *icaD* have been reported to play a significant role in biofilm formation in *S.aureus* and *S.epidermidis* (6, 8, 9). The *icaA* gene encodes N-acetylglucosaminyltransferase, the enzyme involved in the synthesis of N-acetylglu-

cosamine oligomers from UDP-N-acetylglucosamine. Further, *icaD* has been reported to play a critical role in the maximal expression of N-acetylglucosaminyltransferase, leading to the phenotypic expression of the capsular polysaccharide (6-9).

The present study was carried out to determine the slime and biofilm producing ability as well as the presence of the *icaAD* gene in *S.aureus* strains from wound infection.

Materials and Methods

Bacterial strains Fifty strains isolated from wound infections were initially identified by colonial appearance, morphology, positive coagulase and DNase tests. Bacteria were maintained in trypticase soy broth (TSB), to which 15% glycerol was added, at -80 °C.

Slime production assay Slime production assay was performed by cultivation of the *S. aureus* strains on CRA agar (Congo Red Agar) plates (9).

CRA plate (0.8g of CR and 36 g saccharose to 1 liter of brain heart infusion agar) were incubated for 24-48 h at 37 °C and subsequent storage at room temperature for 48-72h. Since the onset of black coloration, occurs earlier for *S. epidermidis* than for *S.aureus*, colonies on CRA were kept under observation for up to 72h.

Slime producing strains form black colonies, whereas nonproducing strains develop red colonies.

Biofilm assay The ability of *S.aureus* isolates to produce biofilm was performed as described previously (10). *S.aureus* strains were grown overnight in TSB with 0.25% glucose at 37 °C. The next day, the strains were diluted 1/200 in TSB with 0.25% glucose and 200 µl of this suspension was inoculated in triplicate to polystyrol microtiter plates and incubated overnight at 37 °C. Then, the cells were decanted, and wells were washed three times with PBS, dried at room temperature and finally stained with 0.1% safranin.

Absorbance of the adherent cells was measured at 490 nm using a microplate reader. A strain was considered positive, if its absorbance value was >0.12.

PCR method for amplification of *icaAD* gene

The chromosomal DNA from the *S.aureus* was extracted as described previously (9). Primers used to amplify a 407 bp product from *icaAD* gene were designed from the published gene bank sequences (locus AF086783). Forward primer: (5'-TATTCAATTACAGTCGCAC-3') and reverse primer: (5'-GATTCTCTCCCTCTCTGCCA-3').

A 25µl PCR mixture contained 2.5mM MgCl₂, 1 U of Taq DNA polymerase, 100µM of each dNTPs, 1µM of each primers and 200ng of DNA sample.

PCR amplification was performed with DNA Thermal cycler (TECHNE, England) and cycling conditions for the amplification of *icaAD* included an initial denaturation step (2 min at 94 °C) followed by 30 cycles of amplification (denaturation at 94 °C for 30s, annealing at 58° C for 30s and elongation at 72 °C for 30s) terminated with a 3 min incubation step at 72 °C. After amplification, 10 µl of PCR mixture was analyzed by 2% agarose gel electrophoresis. The Gene Ruler 100bp DNA ladder plus (Fermentas,) was as a DNA size marker.

Results

Slime producing strains appeared as rough, dry and black colonies, and non-slime -producing strains as pinkish red and smooth colonies.

Twenty-seven of 50 *S.aureus* strains (54%) were slim-producing and 23 of 50 strains (46%) produced smooth and red colonies. Quantitative biofilm production was determined by microtiter plate assay.

Fifty strains were identified, of them 26 (52%) were positive biofilm forming, and 24(48%) isolates were negative. Two strains produced black colony on CRA plate, were biofilm negative on microtiter plate.

The PCR method was applied to 50 strains. As shown in Fig. 1, all the *S.aureus* isolates was positive for icaAD gene. A 407 bp band were obtained in slime and nonslime producing strains.

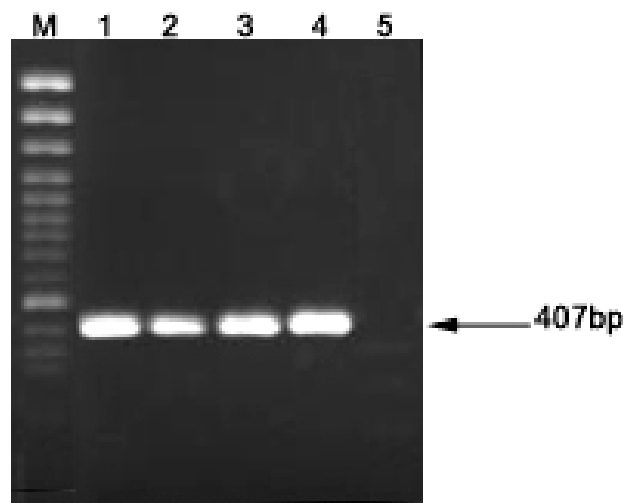


Fig. 1: PCR results with primers for icaAD gene. M: Molecular size marker; lane 1, positive control; lane 2,3,4, 407bp band obtained with DNA from biofilm forming and non biofilm forming strains ; lane 5, Negative control.

Discussion

The Pathogenesis of a particular *S.aureus* strain is attributed to a combination of extracellular factors and properties such as adherence and biofilm formation (5, 7). Wound surface provides an ideal environment for bacteria, where it attaches, grows and remains component of an early biofilm.

The ability of *S.aureus* to form biofilms helps the bacterium to survive hostile environment within the host, and is considered responsible for chronic or persistent infections (8, 11). Several studies have shown that formation of slime and biofilm in *S.aureus* and *S.epidermidis* causing catheter associated and nosocomial infections is associated with the presence of icaA and icaD genes (9,12).

In the present study, although only 54% of the strains produced biofilm invitro, but all the 50

isolates demonstrated icaAD gene. Similarly, Cramton et al. and Fouler et al. observed that icaA was present in all *S.aureus* strains studied (7, 13). By contrast, Arciola et al. observed that 14 out of 23 *S.aureus* isolates from catheter-associated infections and 11 of 15 isolates from periporosthetic infections in human were slime producers (9).

This study indicates a high prevalence of the icaAD gene among *S.aureus* isolates, and their presence is not always associated with invitro formation of slime or biofilm. Since, biofilm protects microorganisms from opsonophagocytosis and antimicrobial agent as well as has a direct and indirect effect on wound healing process, through the production of destructive enzymes and toxin and promoting a chronic inflammatory state (14-16), it is important to diagnose rapidly these strains and to administer prophylactic antibiotic just before and during the surgical procedure for eliminating planktonic bacteria before they can form a biofilm.

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